Ammodytoxin, a neurotoxic snake venom sPLA₂, binds specifically to cytochrome c oxidase in mitochondria of target cells

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Ammodytoxin (Atx) is a presynaptically neurotoxic secreted phospholipase A_2 (sPLA₂) from the venom of the nose-horned viper (Vipera a. ammodytes). The exact mechanism of its neurotoxicity is still unknown however it seems to be also intracellular. One of the intracellular high affinity acceptors of Atx is an integral mitochondrial membrane protein of 25 kDa, designated R25¹. Crude mitochondrial-synaptosomal fraction of porcine cerebral cortex was solubilized and separated on Atx-affinity chromatography followed by SDS-PAGE. The band at 25 kDa was excised and analyzed by mass spectrometry, which revealed the identity of this Atx-binding protein as the subunit II of cytochrome c oxidase (CCOX), the terminal oxidase in the respiratory chain. The specific interaction between Atx and CCOX was confirmed by affinity-labelling procedures. To assess the physiological significance of the Atx-CCOX interaction. NGFdifferentiated PC12 cells were incubated in the presence of a biotin-containing derivative of AtxC. The complexes of AtxC and its binding proteins were purified in two consecutive affinity chromatography steps and detected using streptavidin-HRP. The intensity of the band, representing the subunit II of CCOX, correlated with the duration of the incubation of the cells with AtxC. Using fluorescent confocal microscopy, a substantial co-localization of the two proteins in NGF-differentiated PC12 cells was demonstrated. The extent of co-localization was increasing with the time of incubation in line with the photo cross-linking results. Altogether, our results strongly suggest that the two proteins interact also in vivo and that their interaction is relevant for the observed pathology of neuronal cells caused by Atx.

Reference:

1. J. Šribar, A. Čopič, M. Poljšak-Prijatelj, J. Kuret, U. Logonder, F. Gubenšek, I. Križaj, *FEBS Letters* **2003**, 553, 309–314.